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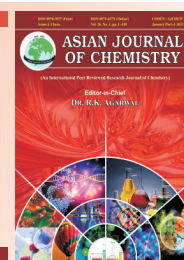


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Antifungal and Bioherbicidal Properties of Essential Oils of *Thymus fallax* Fish & Mey., *Origanum vulgare* L. and *Mentha dumetorum* Schult.

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Received: 25 October 2013;

Accepted: 28 February 2014;

Published online: 28 July 2014;

AJC-15652

The chemical composition of the essential oils obtained from the aerial parts of *Thymus fallax*, *Origanum vulgare* and *Mentha dumetorum* was analyzed by gas chromatography-mass spectrometry and the following were found to be the main constituents: *T. fallax*-thymol (41.48 %), *o*-cymene (26.75 %), ζ -terpinen (15.84 %), 2-isopropyl-1-methoxy-4-methylbenzene (5.10 %), terpineolene (2.11 %) and carvacrol (1.28 %); *O. vulgare*-thymol (50.41 %), carvacrol (12.96 %), 2-bornene (11.28 %), ζ -terpinen (8.80 %), *o*-cymene (6.68 %), α -bisabolane (2.19 %) and caryophyllene (1.31 %); and *M. dumetorum*-carvone (39.64 %), eucalyptol (14.34 %), dihydrocarvone (12.78 %), limonene (7.79 %). The antifungal activities of the oils against *Alternaria solani*, *Fusarium oxysporum* and *Rhizoctonia solani* were also evaluated and were found to be toxic to the pathogens. The results revealed that essential oils, especially those of *T. fallax* and *O. vulgare*, had a strong antifungal activity with a significant inhibition on the growth of the 3 tested fungi. In contrast, the *M. dumetorum* oil did not inhibit the growth of *Rhizoctonia solani* and also exerted a limited inhibitory effect on the mycelial growth of the other two fungi tested. The results of herbicidal assays using these essential oils against four different plant species, *Abutilon theophrasti* Medik., *Agrostemma githago* L., *Medicago sativa* L. and *Lepidium sativum* L., showed that the oils had inhibitory effects on seed germination and seedling growth. The findings of the present study confirmed that plant essential oils can be used as natural herbicides and fungicides to control weeds and pathogenic fungi, thus, reducing the dependence on synthetic pesticides.

Keywords: *Thymus fallax*, *Origanum vulgare*, *Mentha dumetorum*, Essential oils, Biological activities.

INTRODUCTION

Recently, much research has been conducted on the increased food production that will be needed for the rapidly increasing world population and on synthetic pesticides, with the goal of reducing damage to the environment and human health.

Unfortunately, substantial yield losses occur due to insects and plant diseases caused by fungi, bacteria and viruses^{1,2}. Synthetic chemicals (*e.g.*, herbicides, fungicides and insecticides) are widely used in the control of plant diseases, pests and weeds. However, these chemicals may cause toxic residues in treated products³. As mentioned above, synthetic pesticides can also cause environmental pollution owing to their slow biological disruption^{4,5}. In addition, other disadvantages of synthetic pesticide usage are the risk of resistance development by microorganisms, weeds and insects and the high cost of the products⁶⁻⁸.

Another major problem in world agriculture is the losses in crop yield caused by weeds; as a control measure, farmers have commonly applied herbicides to their crops. However, the wide use of synthetic herbicides has been demonstrated to cause pollution in soil and groundwater and lead to the development of weed resistance^{8,9}. Furthermore, herbicides at high concentrations can also increase the risk of toxic residues in agricultural products. Therefore, scientists have searched for natural substances that have different and selective herbicidal mechanisms in comparison to their synthetic counterparts⁹⁻¹².

In Turkey, aromatic plants are widely distributed and there are very rich and diversified floras and many of these plants have been recognized for their nutritional and medicinal characteristics. In Turkey, approximately 140 medical plants have been reported on to date. These plants are used in various industries, such as cosmetics, perfumes, detergents, pharmacology and food flavoring. However, a newly developing industry

may be added to these traditional sectors *i.e.*, the plant protection industry^{13,14}.

The family Lamiaceae (Labiatae) is represented in Turkey by 46 genera and 571 species, of which 44.2 % are endemic; including subspecies, varieties and hybrids, a total of 763 taxa exist. *Thymus*, *Origanum* and *Mentha* are well known genera in the Lamiaceae family¹⁵ and these genera have generally been used as traditional remedies to treat various ailments. For example, they are used as expectorant carminatives and aromatics to relieve whooping and convulsive coughs, digestive disorders and menstrual problems and as anesthetics, antiseptics, abortifacients and antirheumatics. Additionally, they can be used as antimicrobials, insecticides, antifungals and herbicides repellents^{11,16-21}.

The objective of this study was to assess, the antifungal and bioherbicidal effects of essential oils on some pathogenic fungi and plant species. The toxicities of the volatile essential oils obtained from three plant species *i.e.*, *Thymus fallax*, *Origanum vulgare* and *Mentha dumetorum*, were used in tests on plants (*Abutilon theophrasti*, *Agrostemma githago*, *Medicago sativa* and *Lepidium sativum*) and three plant pathogenic fungi (*Alternaria solani*, *Fusarium oxysporum*, *Rhizoctonia solani*).

EXPERIMENTAL

Plant material and the isolation of essential oils *Thymus fallax* and *Origanum vulgare*: The experimental plants were collected from Ordu/Turkey in July 2009 and were confirmed by Prof. Dr. Hamdi G. Kutbay, Department of Biology, Faculty of Science and Art, Ondokuz Mayıs University. *Mentha dumetorum* was harvested from the Gaziosmanpasa University Agricultural Faculty test area in May of 2010. The essential oils were isolated from the plant materials using a water distillation technique *via* a Neo-Clevenger type apparatus. For the extraction of the volatile compounds, the plant materials were weighed (100 g), 400 mL deionizer water was added and the distillation process was continued for approximately 2 h. The essential oils were separated and dried with anhydrous Na₂SO₄ and stored in dark bottles at 4 °C until use and analysis.

Gas chromatography-mass spectrometry analysis: Gas chromatographic (GC) analyses were performed using a Perkin Elmer Clarus 500 Series GC system, in split mode, 50:1, equipped with a flame ionization detector (FID) and a mass spectrometer (MS) equipped BPX-5 apolar capillary column (30 m × 0.25 mm and 0.25 m ID). Helium (1 mL/min) was used as the carrier gas. The injector temperature was set at 250 °C and the FID was operated at 250 °C. An initial column oven temperature of 50 °C was elevated to 220 °C at a rate of 8 °C/min and then held for 5 min. The mass spectrometer conditions were as follows: Transfer line temperature at 250 °C, ion source at 250 °C and the ionization energy at 70 eV. The standard components were available for the majority of the essential oil constituents and Kovats retention indices were determined for all of the sample components using the Van den Dool and Kratz equation according to the homologous *n*-alkane series retention times. Two MS libraries were used to confirm the identities of the compounds: Wiley MS Library and National Institute of Standards and Technology (NIST). Identification of the oil components was accomplished based on a comparison of their retention times with those of authentic

standards (co-injection) and by a comparison of their mass spectral fragmentation patterns. The relative peak-area percentages of the compounds were calculated based on the FID data.

Antifungal activity assays: These assays were carried out to determine the effects of *T. fallax*, *O. vulgare* and *M. dumetorum* against *A. solani*, *F. oxysporum* and *R. solani*. The fungi were obtained from the culture collection at the Faculty of Agriculture, Department of Plant Protection at Gaziosmanpasa University, Tokat. The antifungal activity was studied using a contact assay (*in vitro*) that produces hyphal growth inhibition⁸. Sterile potato dextrose agar (PDA) was cooled in a water bath at 40 °C and the essential oils were mixed with the sterile PDA to obtain final concentrations of 125, 250, 500 and 1000 ppm. The PDA was poured into 90-mm Petri plates (15 mL plate⁻¹). Then a agar disc (5 mm in diameter) of *A. solani*, *F. oxysporum* and *R. solani* were inoculated on the medium and the plates were incubated for 7 days at 25 °C. PDA without essential oils was used as a negative control and synthetic Maneb fungicide (0.4 g/200 mL PDA) was used as a positive control. The experimental design was a randomized block design with four replications per treatment. The radial growth of the fungi was recorded after 7 days.

The growth inhibition was calculated as the percentage of inhibition of radial growth relative to the control using the following equation⁸:

$$\text{Inhibition (\%)} = 100 \times (C - T)/C$$

where C represents the mean of three replicates of hyphal extension (mm) of the controls and T is the mean of three replicates of hyphal extension (mm) of the plates treated with the essential oil. The experiment was repeated twice.

Seed germination and seedling growth experiments: The experiments were conducted in Petri dishes (60-mm diameter) containing two layers of filter paper. Depending on the species (*i.e.*, *Abutilon theophrasti*, *Agrostemma githago*, *Medicago sativa* and *Lepidium sativum*), 15-25 seeds were homogeneously placed in each Petri dish and the dishes were watered using distilled water. Since essential oils have a low solubility in water, they were used in the gas phase. A given volume of each oil was placed on a piece of filter paper that was glued to the inside cover of each Petri dish¹¹. The cover was closed and immediately sealed with parafilm. By using a micropipette, doses of 0 (control), 2, 5, 10 or 15 µL/petri dish were applied. The experiments were conducted in four replicates. Petri dishes were incubated at an average temperature of 24 °C for 1 to 2 weeks, depending on the weed species. After the end of incubation period, the number of germinated seeds and seedling lengths were measured. The experiments were repeated twice.

Statistical analyses: The data were analyzed using the Analysis of variance (ANOVA) test. The means of treatments were grouped on the basis of least significant difference (LSD) at the 0.05 probability level. The SAS software was used to conduct all statistical analyses.

RESULTS AND DISCUSSION

The compositions of the volatile oils extracted by hydro-distillation from the aerial part of the plants are reported in Table-1, together with the Kovats' Indices (KI) calculated for

each compound, the per cent composition and the identification methods. Approximately 24 (94.86 % of the total oil), 19 (98.26 % of the total oil) and 17 (97.51 % of the total oil) constituents were identified from the *Mentha dumetorum*, *Thymus fallax* and *Origanum vulgare* essential oils, respectively. The volatile compounds of *M. dumetorum* were found to be rich in carvone (39.69 %), eucalyptol (14.34 %), dihydrocarvone (12.78 %) and limonene (7.79 %). The *T. fallax* essential oils were found to be rich in thymol (41.48 %), *o*-cymene (26.75 %), ζ -terpinen (15.84 %) and 2-isopropyl-1-methoxy-4-methyl benzene (5.10 %), whereas the *O. vulgare* essential oil was rich in thymol (50.41 %), carvacrol (12.96 %), 2-bornene (11.28 %), ζ -terpinen (8.80 %) and *o*-cymene (6.68 %). The GC-MS analysis of the oils showed an abundance of oxygenated monoterpenes in all of the plants (76.35, 48.44 and 64.69 %, respectively, for *M. dumetorum*, *T. fallax* and *O. vulgare*). The monoterpene contents were 10.2, 48.57 and 29.32 % for *M. dumetorum*, *T. fallax* and *O. vulgare*, respectively.

TABLE-1
ESSENTIAL OIL CONTENTS OF *M. dumetorum* (MD), *T. fallax* (TF) AND *O. vulgare* (OV) PLANTS

RI*	Compounds	MD	TF	OV	Identification technique
953	α -Thujene	~**	0.95	tr***	MS, RI
965	Camphene	-	0.11	-	MS, RI
988	α -Pinene	0.41	0.45	0.73	MS, RI
990	β -Thujene	0.23	-	-	MS, RI
999	β -Pinene	1.05	0.95	0.54	MS, RI
1013	3-carene	0.20	0.68	-	MS, RI
1022	α -phellandrene	0.52	0.22	tr	MS, RI
1033	Terpineolene	-	2.11	0.87	MS, RI
1043	<i>o</i> -cymene	-	26.75	6.68	MS, RI
1048	Linalol formate	1.46	-	-	MS, RI
1067	3-octanol	0.21	-	-	MS, RI
1074	ζ -Terpinen	-	15.84	8.80	MS, RI
1102	Limonene	7.79	0.36	0.42	Co-injection
1110	Eucalyptol	14.34	0.14	tr	Co-injection
1247	Thymolmethyleter	-	-	0.94	MS, RI
1257	2-Isopropyl-1-methoxy-4-methyl benzene	-	5.10	0.38	MS
1271	Borneol	1.31	0.11	tr	Co-injection
1275	4-Terpineol	0.47	-	-	Co-injection
1294	Dihydrocarvone	12.78	-	-	MS
1316	Thymol	-	41.48	50.41	Co-injection, NMR
1322	Carvacrol	-	1.28	12.96	Co-injection, NMR
1339	Isopulegone	0.53	-	-	MS, RI
1348	Carvone	39.64	0.33	-	Co-injection
1365	2-Bornene	-	0.15	11.28	MS, RI
1379	Isobornylacetate	0.32	-	-	MS, RI
1418	Dihydrocarveol	5.32	-	-	MS, RI
1481	α -Bourbonene	3.57	-	-	MS, RI
1501	Methyl-eugenol	0.21	-	-	Co-injection
1521	Caryophyllene	1.73	1.06	1.31	Co-injection
1525	α -bisabolane	tr	0.20	2.19	MS, RI
1532	α -cubebene	0.27	-	-	MS, RI
1548	Germacrene	0.50	tr	-	MS, RI
1572	Isoledene	0.55	-	-	MS
1596	Copaene	1.67	-	-	MS
	Monoterpenes	10.2	48.57	29.32	
	Oxygenated monoterpenes	76.35	48.44	64.69	
	Sesquiterpenes	8.29	1.26	3.5	
	Total	94.86	98.27	97.51	

RI: Retention index, tr: < 0,05 %, nd: not detected; MS: Mass spectrophotometer

Antifungal activity of essential oils: The results obtained in the antifungal activity assays of the essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* against 3 agriculturally important fungal species are shown in Tables 2-4 and Figs. 1-3.

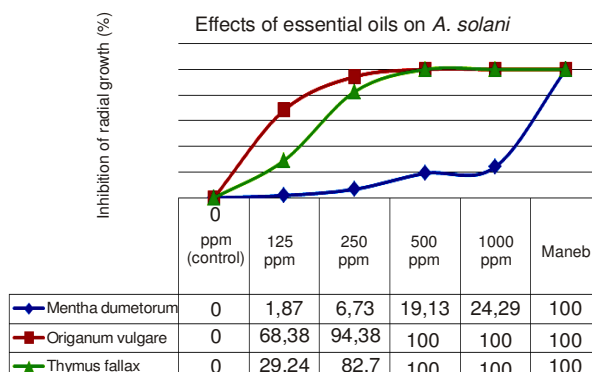


Fig. 1. Inhibitory effects on radial growth rates of essential oils on *A. solani*

TABLE-2
INHIBITORY EFFECTS OF ESSENTIAL OILS ON *A. solani*

Treatments (ppm)	Essential oils		
	<i>Mentha dumetorum</i>	<i>Origanum vulgare</i>	<i>Thymus fallax</i>
Control	0.00 a ^a (42.50) ^b	0.00 a (42.50)	0.00 a (42.50)
125	1.87 b (41.70)	68.38 b (13.43)	29.24 b (30.06)
250	6.73 c (39.63)	94.38 c (2.87)	82.70 c (7.34)
500	19.13 d (34.36)	100.00 c (0.00)	100.00 c (0.00)
1000	24.29 e (32.17)	100.00 c (0.00)	100.00 c (0.00)
Maneb	100.00 f (0.00)	100.00 c (0.00)	100.00 c (0.00)
LSD	3.44	7.30	6.26

^aMeans in the same column with the same letter were not significantly different by ANOVA ($\alpha = 0.05$); ^bRadial growth after 7 days (mm)

TABLE-3
INHIBITORY EFFECTS OF ESSENTIAL OILS ON *F. oxysporum*

Treatments (ppm)	Essential oils		
	<i>Mentha dumetorum</i>	<i>Origanum vulgare</i>	<i>Thymus fallax</i>
Control	0.00 a ^a (42.50) ^b	0.00 a (42.50)	0.00 a (42.50)
125	0.00 a (42.50)	41.26 b (24.95)	0.00 a (42.50)
250	12.24 c (39.99)	63.71 c (15.41)	60.65 b (16.71)
500	8.33 bc (38.95)	95.96 d (1.71)	98.03 c (0.83)
1000	5.89 b (37.29)	100.00 d (0.00)	100.00 c (0.00)
Maneb	54.63 d (19.27)	54.63 c (19.27)	19.27 b (19.27)
LSD	4.36	9.24	3.72

^aMeans in the same column with the same letter were not significantly different by ANOVA ($\alpha = 0.05$); ^bRadial growth after 7 days (mm)

TABLE-4
INHIBITORY EFFECTS OF ESSENTIAL OILS ON *R. solani*

Treatments (ppm)	Essential oils		
	<i>Mentha dumetorum</i>	<i>Origanum vulgare</i>	<i>Thymus fallax</i>
Control	0.00 a ^a (42.50) ^b	0.00 a (42.50)	0.00 a (42.50)
125	0.00 a (42.50)	0.00 a (42.50)	0.00 a (42.50)
250	0.00 a (42.50)	82.90 b (7.26)	0.00 a (42.50)
500	0.00 a (42.50)	100.00 b (0.00)	100.00 b (0.00)
1000	0.00 a (42.50)	100.00 b (0.00)	100.00 b (0.00)
Maneb	100.00 b (0.00)	100.00 b (0.00)	100.00 b (0.00)
LSD	0.00	21.98	0.00

^aMeans in the same column with the same letter were not significantly different by ANOVA ($\alpha = 0.05$); ^bRadial growth after 7 days (mm)

TABLE-5
EFFECTS OF ESSENTIAL OILS ON THE GERMINATION AND ROOT AND SHOOT LENGTHS OF *Abutilon theophrasti* MEDIK. (MD, *M. dumetorum*; TF, *T. fallax*; OV, *O. vulgare*; GR, GERMINATION; RL, ROOT LENGTH; SL, SHOOT LENGTH)

Treatments (E. oils)	2 µL/Petri dish E. oil			5 µL/Petri dish E. oil			10 µL/Petri dish E. oil			15 µL/Petri dish E. oil		
	GR [%]	RL [mm]	SL [mm]	GR [%]	RL [mm]	SL [mm]	GR [%]	RL [mm]	SL [mm]	GR [%]	RL [mm]	SL [mm]
Control	79.9a ^a	18.5 a	16.1a	79.9a	18.5 a	16.1 a	79.9a	18.5	16.1	79.9	18.5	16.1
TF	35.5b	5.9b	4.6b	6.6b	1.9b	0.3b	2.2b	0.0	0.0	0.0	0.0	0.0
OV	2.2c	5.4b	0.0c	0.0b	0.00b	0.0b	0.0b	0.0	0.0	0.0	0.0	0.0
MD	0.0c	0.0b	0.0c	0.0b	0.00b	0.0b	0.0b	0.0	0.0	0.0	0.0	0.0
LSD	17.33	6.049	4.486	10.16	3.198	1.640	7.661	-	-	-	-	-

^aMeans in the same column with the same letter were not significantly different by ANOVA ($\alpha = 0.05$)

As shown in Table-2 and Fig. 1, the essential oil of *M. dumetorum* exhibited an inhibitory effect on the radial growth of *A. solani*. The observed inhibitory effects of *M. dumetorum* at 125-1000 ppm varied between 1.87 and 24.29 %. In addition, the inhibition of the *M. dumetorum* oil on the growth of the tested fungi was significantly lower than Maneb and the oil was not active against *A. solani*. In contrast, *T. fallax* and *O. vulgare* oils at 250, 500 and 1000 ppm inhibited the radial growth of *A. solani* (100 % inhibition) significantly compared with the control and showed a similar effect as Maneb (100 %).

It is speculated that the major components in the essential oils were probably responsible for the antimicrobial activity. As indicated in Table-1, the essential oil of *O. vulgare* contained mainly thymol, carvacrol, 2-bornene, ζ -terpinen and *o*-cymene. Lee et al.² have reported that, the oil from *O. vulgare* inhibited the radial growth of *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Pythium ultimum* and *Rhizoctonia solani* by 55, 78, 70, 93 and 68 %, respectively. The volatile terpenes, such as thymol, carvacrol and *o*-cymene, were thought to be responsible for the antifungal activity of *O. vulgare* oil².

According to the Table-3 and Fig. 2, the inhibitory effect of *M. dumetorum* on the radial growth of *F. oxysporum* ranged from 0.00 to 12.24 % at 125-1000 ppm and showed lower antifungal effects than the other essential oils. Conversely, *T. fallax* and *O. vulgare* at 500 and 1000 ppm showed complete inhibitory effects on the radial growth of *F. oxysporum*, which ranged from 95.96 to 100 and 98.03 to 100 %, respectively. Furthermore, the *T. fallax* and *O. vulgare* essential oils exhibited significantly higher inhibitory effects than Maneb at 250, 500 and 1000 ppm.

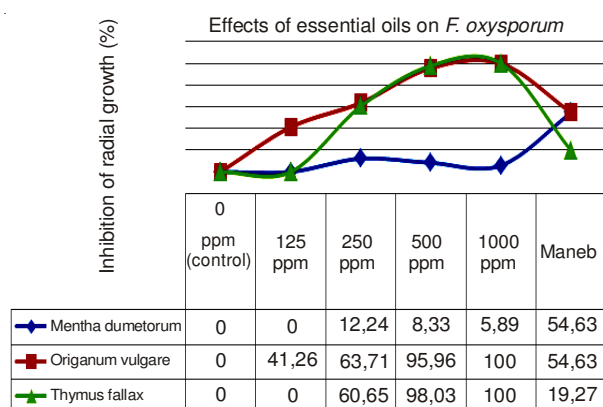


Fig. 2. Inhibitory effects on radial growth rates of essential oils on *F. oxysporum*

According to Table-4 and Fig. 3, the inhibitory effect of *M. dumetorum* on *R. solani* was not statistically significant compared with the control. Conversely, *T. fallax* and *O. vulgare* at 500 and 1000 ppm completely inhibited the radial growth of *R. solani* and the oils of *O. vulgare* and *T. fallax* caused 100 % mycelial growth inhibition.

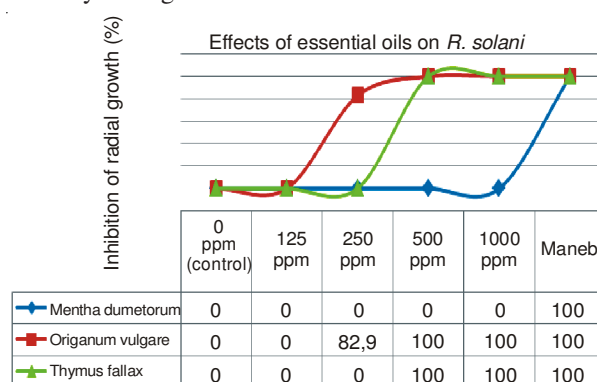


Fig. 3. Inhibitory effects on radial growth rates of essential oils on *R. solani*

In agreement with the results of the present study, Özcan and Boyraz²² have reported that the essential oil of *Origanum vulgare* completely inhibited the mycelial growth of *F. oxysporum*, *R. solani* and *A. solani*. The 10 % level of the oregano decoctions were 100 % inhibitive of mycelial growth in the culture medium at all of the incubation periods.

In previous studies, the methanol extract of *T. fallax* was reported to exert great antimicrobial activity, in particular against *Arthrobacter atrocyaneus*, *Bacillus sphaericus*, *Enterobacter hormaechei*, *Staphylococcus cohnii*, *Pseudomonas syringae* and *Kocuria rosea*. Inhibition of bacterial growth occurred at concentrations ranging from 31.25 to 500 µg/mL²³.

Synthetic fungicides are widely used in the control of plant diseases. These chemicals may cause toxic residues in treated products, environmental pollution and resistance to fungicides among fungal pathogens. Therefore, alternative controls are needed. Because of the low toxicity in mammals, the reduced environmental effect and the wide public acceptance of plant-derived products, researchers have looked to plants for new disease-control agents.

Lee et al.², have defined essential oils as concentrated, hydrophobic liquids containing volatile aromatic compounds extracted from plants, which are rich in bioactive chemicals and may provide potential alternatives to pesticides.

Bioherbicidal effects of the oils: In the present study, the essential oils of *T. fallax*, *O. vulgare* and *M. dumetorum* were tested on the seed germination and seedling growth of *Abutilon theophrasti* Medik., *Agrostemma githago* L.,

Recent studies have shown that both oxygenated monoterpenes and essential oils, which are relatively rich in oxygenated monoterpenes, possess strong inhibitory effects on seed

Essential oils have strong inhibitory effects on the germination and seedling growth of weeds and the mycelium growth of plant pathogenic fungi. Therefore, essential oils are a potential source for the development of new bioherbicides and fungicides. These components should be tested for their bioherbicidal and antifungal activity on different weed species and plant pathogenic fungi.

[illegible]

ACKNOWLEDGEMENTS

The authors are grateful to Prof. Yusuf Yanar, Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, for the help in checking the English.

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